SUMMARY—Studies were made of the influence of heating on nucleotides and total purine nucleosides and bases of beef, pork and lamb muscle. Inosinic acid was the predominant nucleotide in all three species and it was degraded by heating. Adenylic acid increased during cooking in meat from all three species. Cytidylic, uridylic and guanylic acids were present in relatively low concentrations in meat from all three species and changed little during cooking. A rapid method for estimating total nucleotides resulted in greater variation than a specific method for measuring individual nucleotides.

INTRODUCTION

THE FIRST two papers of this series (Macy et al., 1964a, 1964b) outlined the importance of water-soluble constituents as precursors of cooked meat flavor. It was assumed that the constituent molecules or their heat-degraded products acted directly as flavorants of this food.

During the last few years, however, considerable interest has developed in compounds which enhance these flavors. Monosodium glutamate has gained widespread acceptance as a flavor intensifying agent for red meats and chicken. More recently, sodium salts of certain mononucleotides which have a hydroxyl group at position 6 on the purine ring structure have been shown to exhibit a similar effect. The flavor enhancing properties of these mononucleotides (inosinic acid, guanylic acid and xanthylic acids) have been investigated (Wagner et al. 1963).

Kuninaka et al. (1964) reviewed the history and development of nucleotides as food flavoring substances in Japan and further described their properties (Kuninaka, 1966). A synergistic effect with monosodium glutamate was reported. Desirable flavors were enhanced by addition of disodium inosinate, regardless of the type of meat or cookery employed. Disodium inosinate consistently produced an impression of greater viscosity and increased flavor. The basic tastes (sweet, salty, sour and bitter) were not changed in any consistent manner. Suppression of sulfury, fatty, burnt, starchy and hydrolyzed vegetable protein flavors was apparent.

Batzer et al. (1962) found that in addition to glucose and an unidentified glycoprotein, inosinic acid (IMP) was necessary for development of meaty flavor in beef. Nucleotides and nucleosides are also potential precursors of free ribose and ribose phosphate, which have been implicated in Maillard browning reactions.

The objective of these experiments was to determine the stability of certain so called flavor potentiators and related compounds in beef, lamb and pork during heating at various temperatures.

EXPERIMENTAL

Preparation and extraction of beef round roasts

Five U.S. Good grade top rounds from different animals were obtained from a commercial source, excess fat was removed and each divided into three approximately equal parts. Portions from the same relative areas were treated similarly. One portion from each top round was not cooked. Another portion was cooked to an internal temperature of 49°C in a 163°C oven. The remaining portion from each animal was cooked to an internal temperature of 77°C.

Each portion was wrapped in aluminum foil prior to cooking and the exudate collected. Following roasting, the portions were quickly chilled, sliced and ground twice at −3.3°C through a 1/8 in. plate. The juices collected in the aluminum foil during roasting were thoroughly mixed with the respective ground samples prior to the second grinding. The uncooked samples were ground in a similar manner.

Duplicate 30-g samples of each ground roast were homogenized with 50 ml 0.6*N* perchloric acid and filtered. The remaining solids were re-extracted with 50 ml 0.6*N* perchloric acid, filtered and the residues washed with two 10 ml portions of distilled water. The extracts were neutralized with 30% (w/w) potassium hydroxide, the precipitated potassium perchlorate removed by filtration and the filtrates diluted to 150 ml with distilled water. The samples were also analyzed for fat and moisture.

Preparation and extraction of pork loin roasts

Three pork loins were boned out, extraneous fat removed and divided into 3 nearly equal parts. One part of each loin was not cooked. The other 2 parts were wrapped in aluminum foil and roasted in an oven at 163°C. One roast from each loin was roasted to an internal temperature of 49°C while the remaining roast was cooked to an internal temperature of 71°C. Temperatures of the loin roasts were monitored with thermocouples. The tissue was ground and extracted with perchloric acid as described previously for beef.

Preparation and extraction of lamb muscle

Six hanging tenderloin muscles from freshly slaughtered lamb carcasses were divested of as much fat and connective tissue as possible and ground 5 times through a 1/8 in. plate. Eight 20-g samples were packed tightly into 20 × 180 mm test tubes and heated in a water bath at 60°C. Samples were removed at 0, 5, 10, 15, 20, 30, 45 and 60 min and cooled in an ice water bath before extraction. Two 40-ml portions of 0.6*N* perchloric acid were used to extract each samples as described for beef samples.

Ion-exchange chromatography of nucleotides

The method of Lento et al. (1964) as modified by Macy et al. (1966) was used to determine cytidylic, adenylic, uridylic, inosinic and guanylic acids. 30 ml of each extract from beef and pork and 40 ml of the extracts from lamb were analyzed for individual nucleotides.

Quantitation of the results was accomplished by separation and analyses of mixtures of authentic samples of the 5′-isomers of the individual nucleotides. Mixtures containing 0.5, 1.0, 1.5, 2.0 and 2.5 mg of the nucleotides were chromatographed and fractions containing the material for each absorption peak were combined and diluted to 200 ml with distilled water. The absorbance for each compound was then determined at its maximum as follows: cytidylic acid at 280 nm, adenylic acid at 257.5 nm, uridylic acid at 260 nm, inosinic acid at 249.5 nm and guanylic acid at 257 nm.

Absorbance of each compound was a

straight line function of its concentration. Concentrations of the nucleotides in the meat extracts were determined by reference to standard curves following appropriate dilutions.

Absorbance maxima of the individual nucleotides were obtained with a Cary spectrophotometer with authentic samples of the nucleotides.

The absorbance peak for cytidylic acid of the meat extracts contained small amounts of other UV-absorbing materials which was thought to consist mainly of the 2′- and 3′-isomers, and traces of some unknown materials. All of these UV absorbing materials were reported as cytidylic acid.

Analyses of total nucleotides and nucleosides

The batchwise method of Jones et al. (1964) as modified below was used for analyses of total nucleotides and total purine nucleosides and bases.

1 ml of each beef roast extract was mixed with 1-g of damp Dowex 1 × 8 (200-400 mesh) resin (formate form) for at least 15 min with intermittent stirring. The extract and resin were washed quantitatively into a funnel plugged with a small amount of glass wool. The resin, held by the glass wool, was washed with distilled water until a volume of 25 ml was collected. The absorbance of the material washed from the resin was then read at 248 and 260 nm. Total purine nucleosides and bases were calculated from the absorbance at 260 nm of the materials washed from the resin using the molar absorbtivity for hypoxanthine.

Total nucleotides absorbed onto the resin were given by the difference between the absorbances at 248 nm of the resin-treated samples and similar non-treated samples. The molar absorbtivity of inosinic acid was used to calculate total nucleotides.

The method was further modified for the analyses of the pork and lamb samples. The materials washed with water from the ion-exchange columns used for determinations of individual nucleotides were diluted to 500 ml with distilled water and the absorbances determined at 248 and 260 nm. 30 ml of extracts from pork and 40 ml of those from lamb were diluted to 500 ml and the absorbances read at 248 and 260 nm for samples not treated with ion-exchange resin.

Analyses of fat and moisture

These analyses were carried out by the standard methods of the AOAC (1960, sections 22.034 and 22.003).

RESULTS & DISCUSSION

Effects of heating nucleotides of beef round roasts

Mean values and standard deviations of nucleotides of 5 beef round roasts cooked to 49° and 77°C internal temperatures are tabulated in Table 1. Cytidylic acid was not greatly influenced by heating, although the mean for samples roasted to 49°C was lower than that for either the raw samples or those roasted to 77°C. Heating at both temperatures resulted in appreciable increases in adenylic acid. This possibly was due to hydrolysis of adenosine di- and tri-phosphates and pentose nucleic acid.

Table 1—Nucleotide content of raw and roasted beef.[1]

Nucleotide	Internal temperature (°C)		
	Raw	49	77
	mg/100 g dry, fat-free tissue		
Cytidylic acid	13.1 ± 2.0	10.6 ± 2.2	13.0 ± 3.2
Adenylic acid	12.2 ± 3.5	18.1 ± 7.7	41.3 ± 4.1
Uridylic acid	7.1 ± 1.2	4.2 ± 2.9	5.3 ± 2.5
Inosinic acid	278.4 ± 24.6	208.4 ± 40.6	170.3 ± 38.0
Guanylic acid	2.2 ± 4.9	1.2 ± 2.8	3.1 ± 4.6

[1] Results are given as means ±S.D. of 5 top round roasts.

The concentration of uridylic acid was relatively low in all beef samples and it was decreased by heating at both 49° and 77°C. Heating decreased the quantity of inosinic acid in the beef round roast, but not below the quantities necessary for human perception as reported by Wagner et al. (1963) for disodium inosinate. Guanylic acid, another nucleotide in beef roasts with flavor enhancing properties, was present in trace amounts.

The sums of individual nucleotides, total nucleotides (batchwise method) and total purine nucleosides and bases (calculated as hypoxanthine) are presented in Table 2. The sums of the individual nucleotides decreased while the total purine nucleosides and bases increased progressively with increased heating.

Total nucleotide concentrations determined by the batchwise method were similar to the sums of the individual nucleotides in the raw samples, but the total nucleotides increased during heating. This indicated that heating beef produced some materials which absorbed UV light and was adsorbed by the ion-exchange resin, but was not accounted for among the individual nucleotides. The nature of this unknown material was not determined but it was unlikely that it could be di- or tri-phosphonucleotides, as these compounds are easily degraded by heating and heating caused the unknown material to increase.

Effects of cooking on pork loins

To study the changes of nucleotides and related compounds in pork loins during roasting, the data for each cooked sample was divided by the corresponding data for the raw sample from the same loin. These results are tabulated in Table 3.

The influence of roasting on nucleotides of pork was similar to that on beef roasts. All nucleotides decreased during roasting except adenylic acid, which increased in quantity during cooking to 71°C as did total purine nucleosides and bases. The sums of individual nucleotides decreased during heating. Total nucleotides measured by the batchwise method increased during cooking to 49°C then decreased during cooking to 71°C.

Effects of cooking on lamb muscle

Although certain trends were established by the above data for pork, changes during heating at specific temperatures were not ascertained. The heating procedures described above for lamb tissue afforded better control of the cooking temperatures. Table 4 contains data on the influence of heating at 60°C for various lengths of time on nucleotides of ground lamb muscle.

Guanylic and uridylic acids were destroyed after 5 and 15 min heating, respectively. Cytidylic acid increased gradually throughout the heating period

Table 2—Effects of roasting on nucleotides and nucleosides and bases of beef.[1]

Internal temperature (°C)	Sum of individual nucleotides	Total nucleotides[2]	Total nucleosides and bases[2]
	mg/100g dry, fat-free tissue		
Raw	314	315	101
49	243	313	122
77	233	335	159

Results are given as means of 5 top round roasts.

[2] Batchwise method of Jones et al., 1964.

Table 3—Ratios of sums of individual nucleotides, total nucleotides[1] and total purine nucleosides and bases of cooked relative to those of raw pork.

Constituent	Ratio of chemical constituents in cooked and raw pork[2]		
	Raw	Internal temp (°C) 49	71
Cytidylic acid	1.00	0.67	0.58
Adenylic acid	1.00	0.80	1.81
Uridylic acid	1.00	0.83	0.84
Inosinic acid	1.00	0.90	0.78
Guanylic acid	1.00	0.75	0.67
Sum of individual nucleotides	1.00	0.87	0.83
Total nucleotides[1]	1.00	1.81	1.29
Total purine nucleosides and bases[1]	1.00	1.07	1.16

[1] Method of Jones et al., 1964.

[2] Results calculated from averages of duplicate analyses of 3 pork loin roasts at each temperature.

and nearly doubled the initial value at the end of 60 min. Heating caused adenylic acid to double in quantity after 30 min, then to decrease in concentration after 45 and 60 min heating. Apparently, precursors of adenylic acid were present in the tissue up to 30 min heating time, then after their depletion, degradation of adenylic acid occurred. Inosinic acid decreased very rapidly during the first 30 min at 60°C, then increased slightly during the second 30 min heating time.

Data concerning changes in the sums

Table 4—Effects of heating at 60°C on nucleotides of lamb.[1]

Heating time (min)	(μMoles/100 g wet tissue) Cytidylic acid	Adenylic acid	Uridylic acid	Inosinic acid	Guanylic acid
0	9.3	8.6	14.5	209.1	21.8
5	12.7	6.9	13.3	150.8	19.5
10	11.8	7.5	11.7	88.5	—
15	11.1	7.5	12.0	54.0	—
20	13.9	14.1	—	39.9	—
30	13.9	15.6	—	28.1	—
45	15.8	10.7	—	31.3	—
60	17.3	8.6	—	33.3	—

[1] Results are from heating 1 sample for each time period.

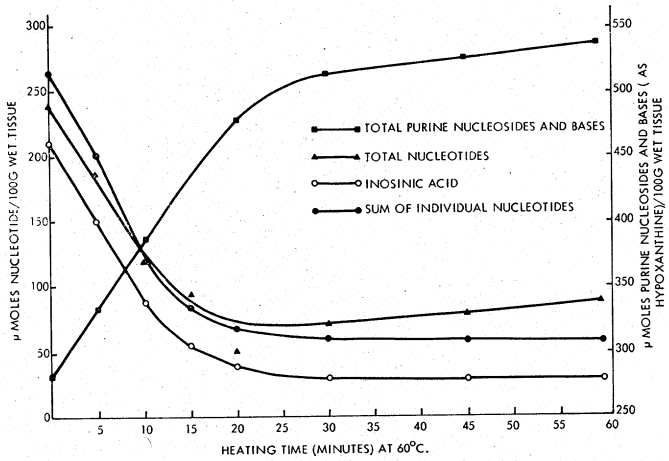

Fig. 1—Effect of heating 60°C on nucleotides and total purine nucleosides and bases of lamb muscle.

of individual nucleotides, total nucleotides, (batchwise method) total purine nucleosides and bases (as hypoxanthine) and the inosinic acid data in Table 4 of lamb muscle are presented in Figure 1. Total nucleosides and bases increased sharply during heating for 30 min, then continued to increase at a much lower rate during the second 30 min heating. Data for sum of individual nucleosides paralleled that of inosinic acid which decreased during the first 30 min heating and remained unchanged thereafter.

Values for total nucleotides (batchwise method) also decreased during the first 20 min heating. After this period, total nucleotides (batchwise method) increased slightly. This confirms that the batchwise method measured UV-absorbing material other than nucleotides and that this unknown material was produced by heating. This unknown material in lamb was qualitatively similar to the unknown UV-absorbing substance found in beef and pork. The increase in total nucleosides and bases during heating was greater than could be accounted for by the decrease in total nucleotides analyzed. This further supports the idea concerning the production during heating of some nonnucleotide base which absorbs at 260 nm.

The data also indicate that heating at 60°C results in rupture of phospho-ester and possibly glycosidic bonds of nucleotides.

REFERENCES

A.O.A.C. 1960. "Official Methods of Analysis," 9th ed. Assoc. Offic. Agr. Chemists. Washington, D.C.

Batzer, O.F., Santoro, A.T. and Landmann, W.A. 1962. Identification of some beef flavor precursors. *J. Agr. Food Chem.* **10,** 94–96.

Jones, N.R. and Murray, J. 1964. Rapid measures of nucleotide dephosphorylation in iced fish muscle. Their value as indices of freshness and of inosine 5′-monophosphate concentration. *J. Sci. Food Agric.* **15,** 684–690.

Kuninaka, A. 1966. Recent studies of 5′-nucleotides as new flavor enhancers. In "Flavor Chemistry" ed. Hornstein, pp. 261–274. American Chemical Society, Washington, D.C.

Kuninaka, A., Kibi, M. and Sakaguchi, K. 1964. History and development of flavor nucleotides. *Food Technol.* **18,** 29.

Lento, H.G., Ford, J.A. and Denton, A.E. 1964. A method for determining 5′-nucleotides. *J. Food Science* **29,** 435–442.

Macy, R.L., Jr., Naumann, H.D. and Bailey, M.E. 1964a. Water-soluble flavor and odor precursors of meat. I. Qualitative study of certain amino acids, carbohydrates nonamino acid nitrogen compounds and phosphoric acid esters of beef, pork, and lamb. *J. Food Science* **29,** 136–141.

Macy, R.L., Jr., Naumann, H.D. and Bailey, M.E. 1964b. Water-soluble flavor and odor precursors of meat. II. Effects of heating on amino nitrogen constituents and carbohydrates in lyophilized diffusates from aqueous extracts of beef, pork and lamb. *J. Food Science* **29,** 142–148.

Macy, R.L., Jr. and Bailey, M.E. 1966. Modified method for rapid determination of individual mononucleotides. *Food Technol.* **20,** 346–347.

Wagner, J.R., Titus, D.S. and Schade, J.E. 1963. New opportunities for flavour modification. *Food Technol.* **17,** 730–735.

Ms. received 12/6/68; revised 5/7/69; accepted 5/8/69.

Contribution from the Missouri Agricultural Experiment Station. Journal Series Number 5545.

A report of work done under contract with U.S. Department of Agriculture and authorized by the Research and Marketing Act of 1946. The contract is being supervised by the Eastern Utilization Research and Development Division of the Agricultural Research Service.